Currently Pending Claims

1. (As Issued) A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display, the zinc finger polypeptide comprising at least one zinc finger having partially randomised allocation of amino acids, the partially randomised zinc finger having a random allocation of amino acids at positions -1, +2, +3 and +6 and at least one of positions +1, +5 or +8, position +1 being the first amino acid in the α-helix of the zinc finger.

2. (As Issued) A library according to claim 1, wherein the partially randomised zinc finger has random allocation of amino acids at each of positions +1, +5 and +8.

3. (As Issued) A library according to claim 1, wherein the encoded partially randomised zinc finger comprises the zinc finger of the Zif 268 polypeptide.

4. (As Issued) A library according to claim 1 as a fusion with a DNA sequence encoding the minor coat protein of bacteriophage λ.

5. (As Issued) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

comparing the binding to one or more DNA triplets of each of a plurality of zinc finger polypeptides having a partially randomized zinc finger, the zinc finger polypeptides being encoded by a library in accordance with claim 1; and

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence.

6. (As Issued) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence, a plurality of zinc finger polypeptides having a partially randomised zinc finger, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the plurality of zinc finger polypeptides being encoded by a library in accordance with claim 1;

comparing the binding to one or more DNA triplets of each of said plurality of zinc finger polypeptides having a partially randomised zinc finger positioned between two or more zinc fingers having defined amino acid sequence; and

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence.

7. (As Issued) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, the method comprising the steps of:

screening against at least a portion of the target DNA sequence, zinc finger polypeptides having a partially randomised zinc finger, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the zinc finger polypeptides being encoded by a library in accordance with claim 1;

comparing the binding to one or more DNA triplets of each of said zinc finger polypeptides having a partially randomised zinc finger;

selecting certain of the screened randomised zinc fingers for analysis of binding characteristics; and

combining those sequences encoding desired zinc fingers to form a sequence encoding a single zinc finger polypeptide.

8. (As Issued) A method for producing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence, zinc finger polypeptides having a partially randomised zinc finger, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the zinc finger polypeptides being coded by a library in accordance with claim 1;

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence; and

expressing the selected nucleic acid sequences to produce zinc finger polypeptides which bind to the target DNA sequence.

9. (As Issued) A library according to claim 1, wherein the zinc finger polypeptide is displayed on a viral particle.

10. (As Issued) A library according to claim 1, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

11. (As Issued) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence, zinc finger polypeptides having a partially randomised zinc finger, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the zinc finger polypeptides being encoded by a library in accordance with claim 1; and

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence.

12. (As Issued) A method according to claim 11, wherein two or more rounds of screening are performed.

13. (As Issued) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, wherein sequences encoding individual zinc fingers

selected by the method of claim 11 are randomly combined in the appropriate order to encode zinc finger polypeptides, the zinc finger polypeptides being screened against the target sequence, that combination of zinc finger sequences encoding a zinc finger polypeptide which binds to the target DNA sequence.

14. (As Issued) A method of modifying a nucleic acid sequence of interest present in a sample mixture by binding thereto a zinc finger polypeptide, wherein the zinc finger polypeptide is designed in accordance with claim 11, comprising contacting the sample mixture with a zinc finger polypeptide having affinity for at least a portion of the sequence of interest, so as to allow the zinc finger polypeptide to bind specifically to the sequence of interest.

15. (As Issued) A method according to claim 14, further comprising the step of separating the zinc finger polypeptide and the sequence of interest specifically bound thereto, from the rest of the sample.

16. (As Issued) A method according to claim 14, wherein the zinc finger polypeptide is bound to a solid phase support.

17. (As Issued) A method according to claim 14, wherein the presence of the zinc finger polypeptide bound to the sequence of interest is detected by the addition of one or more detection reagents.

18. (As Issued) A method according to claim 14, wherein the DNA sequence of interest is present in an acrylamide or agarose gel matrix, or is present on the surface of a membrane.

19. (As Issued) A DNA library according to claim 1, consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, the library being arranged in twelve sublibraries, wherein for any one sub-library one base in the triplet is defined and the other two bases are randomised.

20. (As Issued) A library according to claim 19 wherein the sequences are biotinylated.

21. (As Issued) A library according to claim 19, wherein the sequences are associated with separation means.

22. (As Issued) A library according to claim 21, wherein the separation means is selected from the group consisting of microtitre plate, magnetic bead, non-magnetic bead, sedimentation particle, and affinity chromatography column.

23. (As Issued) A kit for making a zinc finger polypeptide for binding to a nucleic acid

sequence of interest, comprising: a library of DNA sequences according to claim 1 encoding zinc finger polypeptides into a vector; a vector molecule that accepts one or more sequences from the library; and instructions for use.

24. (As Issued) A kit according to claim 23, wherein the vector directs the expression of the cloned sequences as a single zinc finger polypeptide.

25. (As Issued) A kit according to claim 23, wherein the vector directs the expression of the cloned sequences as a single zinc finger polypeptide displayed on the surface of a viral particle.

26. (As Issued) A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences in accordance with claim 1; and instructions for use.

27. (As Issued) A kit according to claim 26, further comprising a DNA library consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, the library being arranged in twelve sub-libraries, wherein for any one sub-library one base in the triplet is defined and the other two bases are randomized.

28. (As Issued) A kit according to claim 27 further comprising appropriate buffer solutions and/or reagents for detection of bound zinc fingers.

29. (As Issued) A kit according to claim 28 further comprising a vector suitable for accepting one or more sequences selected from the library of DNA sequences encoding zinc fingers.

30. (As Issued) A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display, the zinc finger polypeptide comprising at least one zinc finger having partially randomised allocation of amino acids, the partially randomised zinc finger having a random allocation of amino acids at positions -1, +1, +2, +3 and +6, position +1 being the first amino acid in the .alpha.-helix of the zinc finger.

31. (As Issued) A library according to claim 30, wherein the partially randomised zinc finger further has a random allocation of amino acids at position +5.

32. (As Issued) A library according to claim 31, wherein the zinc finger polypeptide is displayed on a viral particle.

33. (As Issued) A library according to claim 31, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

34. (As Issued) A library according to claim 30, wherein the partially randomised zinc finger further has a random allocation of amino acids at position +8.

35. (As Issued) A library according to claim 34, wherein the zinc finger polypeptide is displayed on a viral particle.

36. (As Issued) A library according to claim 34, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

37. (As Issued) A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display, the zinc finger polypeptide comprising at least one zinc finger having partially randomised allocation of amino acids, the partially randomised zinc finger having a random allocation of amino acids at positions -1, +2, +3, +5 and +6, position +1 being the first amino acid in the α-helix of the zinc finger.

38. (As Issued) A library according to claim 37, wherein the partially randomised zinc finger further has a random allocation of amino acids at position +8.

39. (As Issued) A library according to claim 38, wherein the zinc finger polypeptide is displayed on a viral particle.

40. (As Issued) A library according to claims 38, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

41. (As Issued) A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display, the zinc finger polypeptide comprising at least one zinc finger having partially randomised allocation of amino acids, the partially randomised zinc finger having a random allocation of amino acids at positions -1, +2, +3, +6 and +8, position +1 being the first amino acid in the .alpha.-helix of the zinc finger.

42. (New) A polypeptide comprising a designed zinc finger polypeptide and at least one functional domain.

43. (New) The polypeptide of claim 42, wherein the functional domain comprises an activation domain.

44. (New) The polypeptide of claim 43, wherein the activation domain comprises VP-16.

45. (New) The polypeptide of claim 42, wherein the functional domain comprises a nuclear localization signal.

46. (New) The polypeptide of claim 45, wherein the nuclear localization signal is from the large T antigen of SV40.

47. (New) The polypeptide of claim 42, wherein the functional domain comprises a repression domain.

48. (New) The polypeptide of claim 42, wherein the functional domain comprises an epitope tag.

49. (New) The polypeptide of claim 42, wherein the functional domain comprises an immunoglobulin or fragment thereof.

50. (New) A polynucleotide encoding the polypeptide of claim 42.

51. (New) A polynucleotide encoding the polypeptide of claim 43.

52. (New) A polynucleotide encoding the polypeptide of claim 45.

53. (New) A polynucleotide encoding the polypeptide of claim 47.

54. (New) A method of altering expression of a chromosomal gene in an isolated cell, the method comprising the step of:

contacting a target site in the chromosomal gene with a designed zinc finger protein, thereby altering expression of the chromosomal gene.

55. (New) The method of claim 54, wherein the zinc finger protein further comprises at least one functional domain.

56. (New) The method of claim 54, wherein the altering comprises increasing expression of the chromosomal gene.

57. (New) The method of claim 55, wherein the altering comprises increasing expression of the chromosomal gene.

58. (New) The method of claim 54, wherein the altering comprises reducing expression of the chromosomal gene.

59. (New) The method of claim 55, wherein the altering comprises reducing expression of the chromosomal gene.

60. (New) The method of claim 54, wherein the isolated cell is a mammalian cell.

61. (New) The method of claim 54, wherein the designed zinc finger protein is encoded by a nucleic acid molecule operably linked to a promoter and wherein the nucleic acid molecule expresses the zinc finger protein when administered to the cell.

62. (New) The method of claim 61, wherein the nucleic acid molecule further comprises a polynucleotide sequence encoding a functional domain.

63. (New) The method of claim 55, wherein the functional domain comprises an activation domain.

64. (New) The method of claim 62, wherein the functional domain comprises an activation domain.

65. (New) The method of claim 63, wherein the functional domain is VP-16.

66. (New) The method of claim 64, wherein the functional domain is VP-16.

67. (New) The method of claim 55, wherein the functional domain comprises a nuclear localization signal.

68. (New) The method of claim 62, wherein the functional domain comprises a nuclear localization signal.

69. (New) The method of claim 67, wherein the nuclear localization signal is from the large T antigen of SV40.

70. (New) The method of claim 68, wherein the nuclear localization signal is from the large T antigen of SV40.

71. (New) The method of claim 55, wherein the functional domain comprises a repression domain.

72. (New) The method of claim 62, wherein the functional domain comprises a repression domain.

73. (New) The method of claim 55, wherein the functional domain comprises an epitope tag.

74. (New) The method of claim 62, wherein the functional domain comprises an epitope tag.